THE FINER STRUCTURE OF THE HEART MUSCLE OF THE DOG

By GERTRUDE A. GILLMORE

WITH THREE PLATES

During the winter of 1900–1901, the writer began the investigation of the finer structure of the heart muscle with a view to the elucidation of the relation and meaning of the discs. At that time, with the exception of the papers by MacCallum, little or no detailed work on the finer structure appeared to have been done. One found substantially the same brief descriptions and drawings repeated again and again. From these descriptions little information could be gained beyond that of the shape and anastomosing of the cells, the position of the nuclei, presence of the cell cement, the existence of striations, and the supposed absence of sarcolemma. Even the continuity of Krause's membrane was not generally understood. There seemed, therefore, a need of more detailed work on the finer structure of heart muscle.

The facts here given were substantially worked out during the winter of 1901 while in the department of Histology and Embryology of Cornell University, but were not published.[1] Now, that Heidenhain has published such important results, it is hoped confirmation and comparison of some points will not be unwelcome. After a year's unavoidable delay, the writer, through the kindness of Dr. Whitman, has been able to continue her work at the Marine Biological Laboratory, Wood's Holl, Massachusetts.

The heart assigned for study was that of a dog, with comparative work on the hearts of the cat, sheep, rabbit, frog, necturus, and amphiuma. In this report only the first part of the problem will be treated, *i. e.,* the fine structure of heart muscle as illustrated in the heart of a dog.

The material used was obtained from a young dog instantly killed by an accident. Pieces of the heart wall were put into various

[1] An informal report of the results embodied in this paper was given before the Histological Seminary, Cornell University, May 7, 1901.

fluids: Zenker, Flemming, picro-aceto-sublimate, picric alcohol, 67% alcohol, Perenyi, and others. Of the fixatives used, Flemming and picro-aceto-sublimate have proved the most satisfactory. The material was carefully dehydrated, imbedded in paraffin, and cut into sections of from 3 to 5 microns in thickness. Some of the material was stained with Heidenhain's haematoxylin, and some with Wotter's haematoxylin, first mordanted in vanadium chloride. The latter stain was the more satisfactory. The sections were mordanted for three hours in a solution consisting of two parts of a 10% solution of vanadium chloride and ten parts of a 5% solution of acetate of aluminum. They were then stained for three-quarters of an hour, instead of the longer period recommended in Lee. The material was differentiated in absolute alcohols acidulated with $\frac{1}{2}$%, $\frac{1}{5}$%, $\frac{1}{8}$%, and $\frac{1}{10}$% of hydrochloric acid. While the sections were transferred successively through the four differentiators, they were carefully watched every two minutes. They were then cleared in bergamot oil and mounted in balsam. The new methods recommended in Heidenhain's last paper ('01) have not been used, since much of my material was prepared several months prior to that publication. Any later use is now precluded by his request that no one publish any results obtained on muscle by his new methods until his complete work appears. The effect of iron haematoxylin on muscles is well known. With vanadium chloride and haematoxylin the transverse disc, Krause's membrane, and a dark line crossing the cell cement are stained a bright blue. The sarcolemma is faintly tinted a light blue. Since this stain is much more brilliant than iron haematoxylin and tinges more structures, it has proved the more satisfactory one to use.

In the writer's well-fixed preparations of heart muscle few wide breaks occur in the tissue and these breaks are filled with connective tissues, or capillaries. One does not find the commonly figured wide spaces between the fibers, but a mass of fibrils from the formerly so-called cells, whose existence is now doubted by Ebner, Heidenhain, and others, appear to blend together and form other fibers. (See figs. 1 and 3.) Heidenhain has found the same condition in the human heart ('99). He thinks that one does not find cells anastomosing by lateral branches, but that the "general type of lateral connection is by broad regions of fusion between parallel fibers." From a glance at the right of figs. 1 and 3, one sees that by the confluence

of two masses of fibrils from two adjacent cells a third so-called cell is formed which lies in a line intermediate between the other two cells. Repeatedly these masses of fibrils appear to blend in a plane above or below that on which one's sections were cut. (See fig. 3.) Frequently small breaks occur parallel with the fiber but extending only for a short distance, *i. e.,* between two cement bands. (See figs. 2 and 3.) Evidently these separations between fibrils can hardly indicate cell boundaries, for frequently they are only a few fibrils apart. Often any wide lateral break between cells is two or three fibers apart. But in these spaces several small, more or less parallel breaks may occur. In consequence of these conditions, it seems to the writer difficult, or even impossible, to give distinct lateral boundaries for cells. However from a careful comparison of long and cross sections of the fibers, one usually finds near its center nuclei at more or less regular intervals. Occasionally one finds two nuclei close together. In every case there is some cytoplasm surrounding the ellipsoidal nucleus and extending out from it, often in a more or less cone-shaped mass. (See fig. 2.) The areas enclosed between two cement bands are variable; occasionally they are shorter than the length of a nucleus, usually several times its length. (See the extreme upper and lower portions of fig. 3.) In the dog's heart, the writer has never found the bands less than three or four segments apart. Heidenhain, however ('01), states that in the human heart one sometimes finds one segment (Z–Z)[1] enclosed between two bands. This fact, together with the already observed continuity of the fibrils through the cement—Ebner ('00), Hoyer ('01), Heidenhain ('01), and figure by Szymonowicz ('01)—he thinks argues against the cement bands being considered as distinct cell boundaries. Godlewski ('01) has also come to the conclusion that the cells of the myocardium develop as a syncytium. The writer has also found in the dog's heart indications of this same continuity of the fibrillae. This point will be discussed more fully later when the cement bands are described in detail. The more one studies heart muscle the less does the idea of separate cells seem tenable.

Wherever large or small breaks occur in the muscular tissues of the heart of the dog, cat, sheep, or amphiuma, one finds along the

[1] Throughout this paper the writer will use Heidenhain's nomenclature: $Z =$ Krause's membrane; $q =$ transverse disc; $qh =$ the light area which separates the transverse disc into two parts; $J =$ lateral disc; and $M =$ the middle disc.

edge of the fiber a distinct sarcolemma. The presence of this structure in heart muscle was reported by Hoyer ('01), recently by Heidenhain and others. MacCallum ('97) has described in the human heart a condensation of the sarcoplasm, but does not seem to consider it the same as the sarcolemma of skeletal muscle. He thinks each fibril is surrounded by such a condensation. Sometimes between two adjacent fibrils it is double. It then appears raised in folds. At other times between two fibrils there is a single layer with no wavy outline. Heidenhain, on the other hand, having so recently thoroughly studied the human heart, describes the same structure as a sarcolemma. Where this structure appears in successive parallel breaks, which are only a few fibrils apart, he thinks indicates the presence of partially developed fibers. In the hearts studied by the writer, the sarcolemma appeared as a narrow, usually wavy band of homogeneous, hyaline sarcoplasm. (See figs. 2 and 3.) In this band Krause's membrane terminates. When the sarcolemma is raised in waves *Z* ends in the depressions between the waves. One wave extends from *Z* to *Z*. This arrangement seems to be usually the case, except possibly where the cement intervenes. The height of successive waves, where the discs are in like phases, appears to be the same. In thin sections of the hearts of the dog, sheep, and cat the relation of *Z* to the sarcolemma is very apparent. Where the fibrils border the cytoplasm surrounding the nucleus, one finds again the sarcoplasm raised in arches, at the base of each of which *Z* ends. It is interesting to note that in insects where so much muscular effort is put forth, one finds a sarcolemma like that in the heart muscles of vertebrates. The relation of this structure to the cement bands will be described later.

The cement bands extend occasionally across a fiber, but usually one finds the staircase appearance fully described by Heidenhain ('01). (See figs. 1 and 2.) The steps seem to lie edge to edge and seldom to overlap. They may go up and then down, or each step may be one or several segments higher than the one below. Occasionally there appears to be in the dog's heart some slight variability in the breadth of the bands. Usually they are slightly narrower than the area from *Z* to *Z*, but occasionally as broad. In the same section there is little or no variation in the bands. The number of fibrils which are crossed by one of the steps may vary from those which constitute half or three-quarters of a fiber to a single fibril.

The discs crossing the segments just above or just below a step become continuous with those crossing other fibrils which are crossed by other steps. When the cement bands in the dog's heart are examined more carefully, they appear to be composed of rod-like bodies, each of which lies in the same straight line as a fibril. To the writer each rod seems to be a continuation of a fibril. These rods, as shown in figs. 5*c* and 5*d* (Pl. V), do not extend straight across the band, but often either separate or come close together towards the middle of the cement area. Extending across the center of the cement band is a dark blue line. (See fig. 4.) This line stains as does *Z*. Where small breaks occur between fibrils and cement bands, one often sees such a condition as shown in fig. 5*b*, where Krause's membranes appear to become continuous with this blue line. Where a wide break occurs and the single blue line can be seen, the sarcolemma appears to arch down in the middle of the cement band and the blue line to end in the depression. Again one sometimes finds the cement band crossed by two blue lines which are quite close together. One then finds that both lines appear to terminate in the sarcolemma (see fig. 5*a*), or as already shown in fig. 5*b*, each blue line appears to become continuous with an intermediate disc (*Z*) lying just beyond the cement band and the one nearest on a level with the blue band. From a more careful examination of the band, it would look as if the blue might be produced by the staining of the delicate threads which seem to weave in and out between the rods of which the cement seems to be composed. After comparing the writer's sketches of cement areas with those of Szymonowicz ('01) and MacCallum, it was evident that they resemble more closely the latter author's illustrations of the cement bands in the human heart. He states, however, that he was only able to find in the human heart a single line crossing the cement (MacCallum, '97). At first the writer thought that probably the two blue lines were Krause's membranes, which are supposed to border each side of the cement bands. This explanation seemed probable, since the lines ended in the sarcolemma and stained as did *Z*. But of this point the writer is uncertain. If these lines are Krause's membranes, why does one so frequently find the cell cement crossed in the center by a single blue line? Is this effect caused by a slight obliquity in the cutting of the sections? If this is the case, the obliquity was not apparent. Since these lines both stain and

bear the same relation to the sarcolemma as does *Z*, they would seem to be of the same nature. But are they really the same structure? The writer is confident that in the dog's heart one may find the cement band crossed sometimes by one, and sometimes by two blue lines. What they are it seems impossible to say, especially since the nature of the cement band is not fully understood. MacCallum and Przewoski ('93) consider the bands intercellular bridges. Ebner thinks they are contraction areas; and Heidenhain ('01), in his last article, has attempted to disprove both hypotheses and has claimed that they are growth areas; that there segments are being formed as the heart increases in size. Whatever hypothesis one may hold, it is interesting to note that all the discs between the two cement bands appear to the writer to be in like phases. When the discs appear to change their forms, a cement band intervenes.

From a glance at figs. 4*a* to 4*c*, one sees that all the discs, except perhaps *M*, found in skeletal muscle, occur in the heart muscle of the dog: Krause's membrane (*Z*), the lateral disc (*J*), the transverse disc (*q*), the light area which separates *q* into two parts (*qh*), and possibly the middle disc (*M*), is present. *Z* takes a deep blue stain with haematoxylin, does not seem to vary in thickness, and extends as a continuous membrane not only from fibril to fibril across one fiber, but also appears to cross continuously two or even three and possibly more fibers. It is interesting to examine places where fibrils have been pulling a short distance apart, for there one sees Krause's membrane stretching across the intervening space. The relation of *Z* to the sarcolemma has been dwelt upon. On each side of *Z* is the lateral disc (*J*). It is isotropic, more fragile, and less deeply stained than *q*. It appears a little darker than the ground substance, but lighter than *qh*. It does not stain with haematoxylin. The width of *J* varies greatly, as will be seen from figs. 4*a* and 4*b*, but it never entirely disappears. The transverse disc is anisotropic and stains deeply with haematoxylin. It varies greatly in width and outline. In fig. 4*a* it extends almost the entire length of the segment. The outline in long section is then distinctly that of an oblong. As *J* decreases in size, the edges of *q* round off and one gets the bead-like forms shown in fig. 4*b*. In figs. 4*c* and 4*d*, one sees *q* crossed by the light area, thus forming *qh*. Whether one can consider that in fig. 4*c* the narrow area is *M*, seems difficult to determine if one is unable to stain it. Heidenhain, by his later methods, is able to

color this disc and shows that *M* is present in the human heart muscles. He considers it the complete analogue of *Z*, but more delicate in nature. As *q* grows narrower, *J* increases in width. Heidenhain ('99) thinks that *J* and *q* stand in the closest connection with the function of contraction. The bead-like appearance of *q* he considers due to the contraction of the contour lines. Since the transverse discs crossing one fiber lie in the same straight line as those crossing parallel neighboring fibers, one may often trace row after row of these discs extending straight across the field of the microscope. All parallel fibers do not always show like discs in like phases, as is evident from fig. 3, although they are often in like phases.

Whether the various appearances of the transverse and lateral discs indicate extraction of the stain or whether they indicate phenomena of contraction, the writer does not know. As already stated, the indications are that between two cement bands all like discs show like phases, unless the conditions of the discs on the lower edge of fig. 2 is an exception. If the fibers are continuous and if the phases of the discs indicate contraction phenomena, why does the wave contraction stop at the cement band? Why does the impulse gradually die out?

From what has been said one sees that in the dog's heart, as in the human heart, the fibers are packed close together. Fibrils from adjacent cells blend together to form new fibers; the whole making a complex network. Again, where spaces occur between fibrils, one sees along the fiber's edge a narrow, wavy condensation of sarcoplasm resembling the sarcolemma of insect muscle. In this structure terminates Krause's membrane. Near the center of the fibers at more or less even distances apart lie the nuclei. Occasionally two nuclei lie a short distance apart and are connected by a slender column of cytoplasm. The cement bands resemble a series of blocks crossing the fibers. The distance between the bands and the number of fibrils crossed by a portion of a band, or step, may vary greatly. The cement area appears to be crossed by rods which look as if they were the ends of the fibrils. Through the center of the cement area extends one, or occasionally two, blue lines. These lines give indications of becoming continuous with *Z*. In places, also, these lines appear to end in the sarcolemma as does Krause's membrane. At times, in very thin sections, one gets the appearance of an interlacing network crossing the rods. Between two cement areas all the discs

appear in like phases. The discs which the writer has found present are *Z, J, q,* and *qh*. *M* may be present. *Z,* or Krause's membrane, is of uniform thickness, stretches continuously from fibril to fibril, crossing often more than one fiber, and terminates in the sarcolemma. If the sarcolemma is wavy, *Z* terminates in the depressions between the waves. *J* and *q* vary in size but never entirely disappear; as the one increases, the other decreases in size; frequently *q* is bead-like in form; and it is often crossed by a light area, *qh,* which cuts the transverse disc into two parts. The breadth of *qh* varies. As it increases in width, the parts of the transverse disc become bead-like.

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EXPLANATION OF PLATES

Plate III

Fig. 1. Heart muscle of the dog showing the interblending of fibrils, the relative position and shape of the cement bands, and cross striations.

Fig. 2. Same as fig. 1, but showing the relation of the sarcolemma to the muscle fiber and to Krause's membrane.

Plate IV

Fig. 3. Shows cross striations in detail and their relation to those of neighboring fibers and to the cell cement.

Plate V

Fig. 4. Various appearances of the discs. 4*a*. The segment, *Z–Z*, nearly filled by the transverse disc, *q*. 4*b* and 4*c*. Variation in the relation of the transverse disc to the later discs. Two bead-like forms which *q* may assume. 4*d* and 4*e*. The transverse disc crossed by the light area forming *qh*.

Fig. 5. Cell cement. Relation of blue lines crossing the cement to Krause's membrane. *s*, sarcolemma; *x*, cell cement; *y*, blue lines; *z*, Krause's membrane. 5*a*, cement crossed by one blue line. 5*b*, cement crossed by two blue lines.

Fig. 6. Appearance of the cement band and the relation of the blue lines and the sarcolemma to the band. *x*, rods; *y*, blue lines; *z*, Krause's membrane; *s*, sarcolemma.

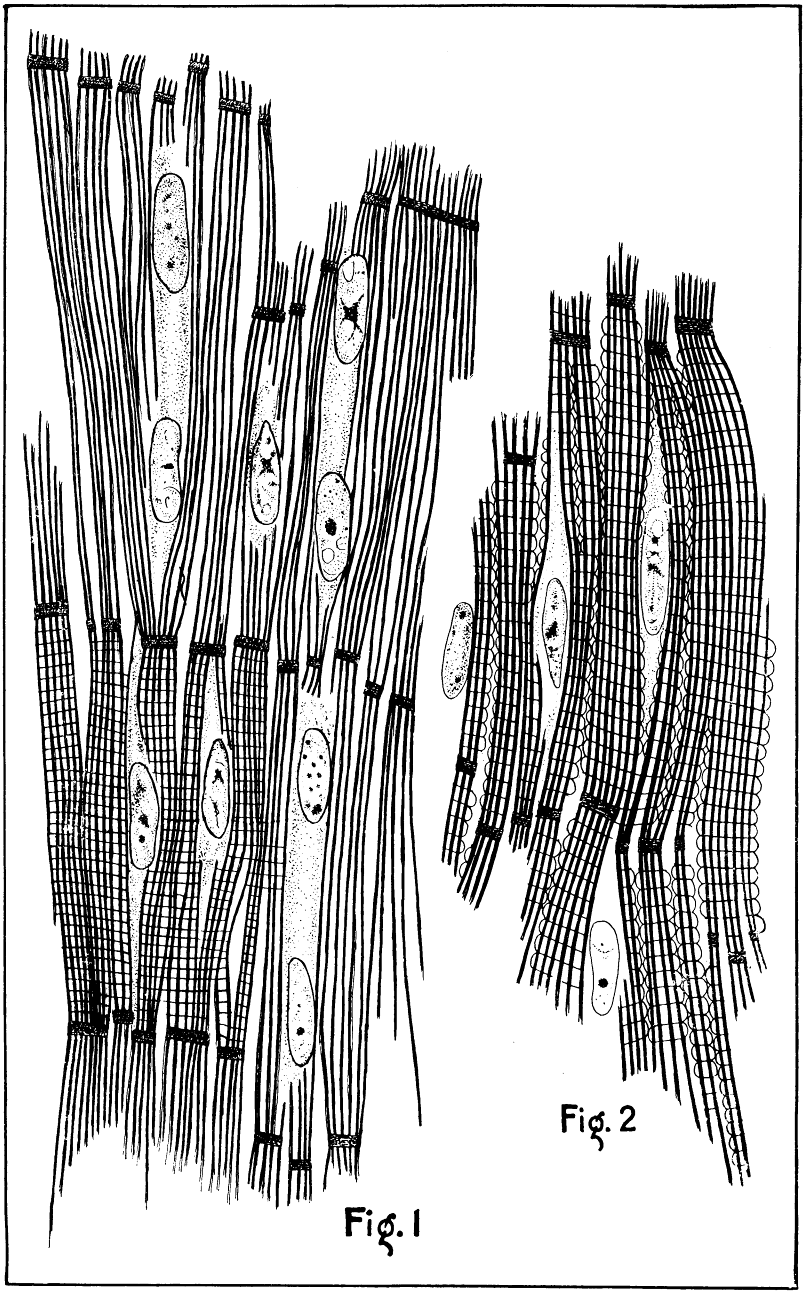


Fig. 1

Fig. 2

PLATE IV

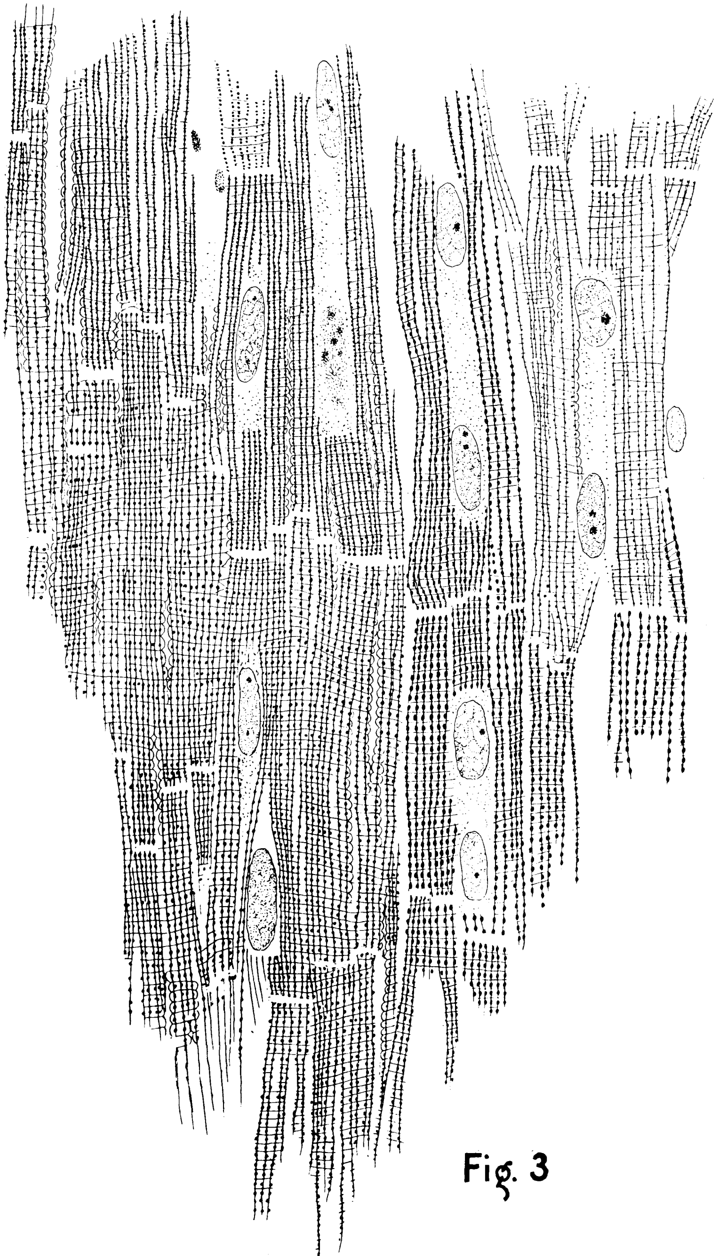


Fig. 3

PLATE V

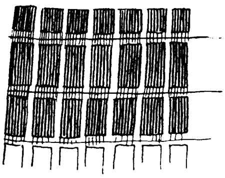


Fig. 4a

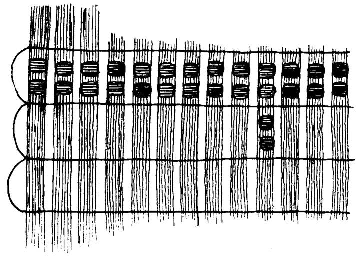


Fig. 4d

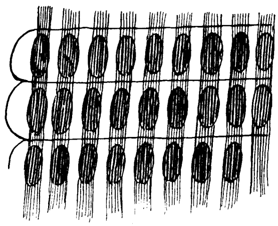
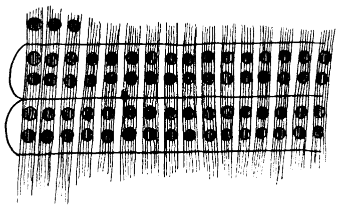


Fig. 4b



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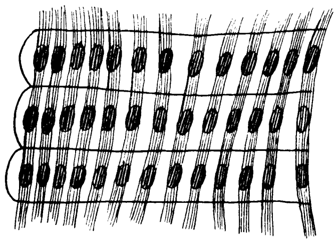

Fig. 4c

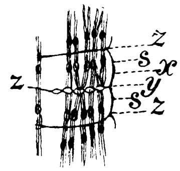

Fig. 6

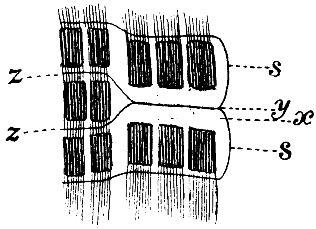

Fig. 5a

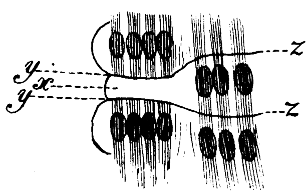

Fig. 5b